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EFFECT OF ADDING NANO-FERTILIZER NPK AND HUMIC ACID ON THE VEGETATIVE GROWTH AND CHEMICAL CHARACTERISTICS OF BITTER ORANGE SEEDLINGS *CITRUS AURANTIUM* L.

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Abstract:

The experiment was conducted in a nursery covered with a shade net at the Department of Horticulture and Landscape Design, Faculty of Agriculture and Wetlands, Dhi Qar University, during the growing season 2021-2022. One-year-old bitter orange variety Citrus aurantium seedlings, which grew as uniformly as possible, were obtained from a nursery in Baghdad Governorate. On February 20, 2022, the seedlings were transplanted into 10 kg plastic pots (26 cm in diameter) filled with a planting medium made of mixed soil and peat moss in a ratio of 1:3. Plant care, including pruning, hoeing, fertilization and watering, was carried out continuously throughout the study period. The study was conducted using a two-factor factorial experiment using a randomized complete block design (R.C.B.D.), in which the first factor was spraying of nanocomposite fertilizer NPK at three concentrations (0, 1, 0.5, 1) g L-1, and the second factor represented spraying of humus at three concentrations (0, 1, 2,) g L-1. Spraying was performed three times every two weeks. The results can be summarized as follows:

Spraying of nanofertilizer NPK showed positive effects on most nutritional and chemical parameters of citrus seedlings, with the 1 g L-1 concentration showing a significant effect and having the highest values in plant height traits, total leaf chlorophyll content, and chlorophyll content. The nitrogen and potassium nutrient contents in the leaves reached 97.47 cm, 26858 mg at 100 g-1 fresh weight, 0.392%, and 1.691%, respectively.

Foliar treatment with humic acid showed positive effects on most nutritional and chemical parameters of orange seedlings of the citrus (Citrus aurantium L) variety. The spray treatment with a concentration of 2 g/L was excellent as it produced the highest plant height values, 11.00 branches in the seedlings, 26.696 mg/100 g/1 fresh weight, and 1.621%, respectively, when the number of branches reached 95.33 cm. The interaction treatments between the two factors examined had positive and significant effects on most of the plant and chemical traits examined. The interaction treatment of Nano-NPK at a concentration of 1 g/L with 2 g/L humic acid achieved the best results on growth indices..

Keywords: Nano-Fertilizer NPK ; Humic Acid ; *Citrus aurantium* L

INTRODUCTION

The bitter orange plant *Citrus aurantium* L. belongs to the citrus genus Citrus, the Rutaceae family, which grows in tropical and subtropical regions. India is considered the original homeland of *Citrus aurantium* (Al-Khafaji et al., 1990). Most citrus species are widespread in the central regions of Iraq due to their suitability to the prevailing environmental conditions. the number of fruit-bearing citrus trees is approximately 7,768,290 million trees, with a production of 176,117 tons (Central Bureau of Statistics, 2020). Citrus fruits are of great importance among other fruit trees due to their nutritional, environmental, economic and medical importance, and consider a rich source of vitamins, especially vitamin C, in addition to being rich in mineral elements necessary to build the human body (Ahmed and Dawoud, 2020).

The foliar fertilization method is efficient and effective in feeding plants through the vegetative parts, as well as providing the plant with nutrients in a homogeneous manner (Brayan, 1999).

It is important to pay attention to adding chemical fertilizers to various plants because of their role in plant growth and development, and even extends to various biological reactions that occur within the plant. excessive use of different chemical fertilizers is considered one of the reasons for environment and soil deterioration. Nano-fertilizers are the latest and most technically advanced in supplying plants with nutrients. mineral and compared to chemical fertilizers and then , the efficiency of fertilizer use improves (Subbarao et al., 2013). where old chemical fertilizers are replaced with nano-fertilizers that are efficient and environmentally friendly in nature, the main use of adding fertilizers is the rapid absorption of nutrients while giving best and fastest yield, the organic matter added to soil improves the physical properties and soil structure, increases the stability of its aggregates and its ability to retain water, and is a source of nutrients necessary for plant nutrition , after added humic acid to the soil the absorption of nutrients by the plant. increases Humic acid is a transport media for nutrients from the soil to the plant, as it affects plant growth through its effect on increasing the growth of the root system (Chen and Aviad, 1990).

Due to the lack of studies on effect of spraying NPK and humic acid on bitter orange seedlings in Dhi Qar Governorate, this study aiming to know the effect of spraying NPK and humic acid individually or in combination in improving the chemical characteristics of bitter orange seedlings with the aim of obtaining strong-growing, viable seedlings to graft them, as well as reduce the environmental and economic impact resulting from using traditional fertilizers.

MATERIALS AND METHODS

1-3: Study location

The experiment was carried out in nursery covered with shade net (saran) belongs to Horticulture and Landscape Department - College of Agriculture and Marshlands at Dhi Qar University during the growing season (2021-2022) where bitter orange seedlings number. *Citrus aurantium* L., were selected one year old, for the purpose of conducting this study. an agricultural growth media consisting of mixed soil and peat moss in a ratio of 1:3 was used, and a random sample was taken from the riverine mixture and analyzed in the laboratories of the Dhi Qar Agriculture Directorate to identify some of the chemical and physical characteristics of the soil, Table (1).

Table (1) Some chemical and physical characteristics of the soil mixture

characteristics	measuring unit	The value
pH	------	7.5
EC	$ds.m^{-1}$	2.51
Soil texture	Silty loam	Mixed sandy
sand	$Mg\ L^{-1}$	70.24
loam	$Mg\ L^{-1}$	14.44
clay	$Mg\ L^{-1}$	15.42
N	$Mg\ L^{-1}$	2.89
P	$Mg\ L^{-1}$	4.55
K	$Mg\ L^{-1}$	6.78

2-3: Preparing and servicing seedlings:

The bitter seedlings were obtained from a nursery in Baghdad Governorate on February 20, 2022. They were one year old, had almost uniform growth, and were planted in polyethylene plastic bags. The seedlings were transplanted into plastic pots with a capacity of 10 kg (26 cm in diameter). Throughout the study period, the plant care process was carried out in a symmetrical manner, including pruning, weeding, fertilization, and watering..

3-3: STUDY PARAMETERS:

The first factor

It included spraying the shoots of seedlings with the nano-compound NPK at three concentrations (0, 0.5, and 1) g L^{-1}.

The second factor

It includes spraying the shoots of seedlings with humic acid at three concentrations: 0, 1, and 2 g L^{-1}.

Studied traits:

Vegetative growth indicators

Vegetative growth measurements were carried out from each experimental unit and included the following:

1-Seedling height (cm):

Measure the plant height from the area where the stem connects to the planting media to the growing tip with a metric ruler.

2- Branches Number (seedling $branch^{-1}$):

Calculate the number of side branches in one plant by counting branches number on the main stem and then extracting each treatment rate.

3- Estimating the total content of chlorophyll in leaves (mg 100 g^{-1} fresh weight):

The total chlorophyll content of leaves was estimated based on (Dere, S., Gunes 1998) method by taking 1 g of fresh leaves and crushing them in a ceramic mortar with 10 ml of acetone its concentration 80%, the filtrate was separated using a centrifuge at a speed of 3000 RPM for 15 minutes. , separating the filtrate process was repeated several times until the green dye disappeared from the precipitate, after that, the optical density of the filtrate was measured using a spectrophotometer at two wavelengths (645 and 663 nm).

4- Nitrogen in leaves (%)

The total nitrogen content of leaves was estimated using a Micro Kjeldahl device and using the method described by Page et al. (1982), by taking 10 ml of digested sample and adding 10 ml of sodium hydroxide (NaOH) 40% concentration ,then a distillation process was carried out, and the liberated ammonia was collected in a glass beaker which contains a mixture of 10 ml of boric acid concentration 2% with the indicators Methyl Red and Bromocresal Green, the collected ammonia with hydrochloric acid (HCl) concentration (0.005).was pulverized After knowing the amount of pulverized HCl acid, the percentage of nitrogen was calculated according to the following equation:

$$N\% = \frac{Volum\ of\ acid\ used\ for\ titration\ X\ \ Acid\ Normality\ \ X\ \ 14\ X\ \ \ volum\ of\ diulotion}{Volum\ of\ sample\ taken\ at\ distilation\ \ X\ \ weight\ of\ digestions\ sample\ \ X\ 1000}\ \ X\ \ 100$$

5-Potassium in leaves (%)

Potassium was estimated after diluting the digestion solution with distilled water, using a JEN WAYPFP 7 flame photometer, and potassium chloride was used to prepare standard solutions to create a standard curve for potassium (Page et al., 1982).

4: RESULTS AND DISCUSSION

1- Seedling height (cm):

The results of Table (2) indicate that the study factors and their interactions which increasing bitter orange seedlings height, spraying the seedlings with nano-NPK had a significant effect on increasing seedlings height with increasing spray concentration, as the spraying treatment with a concentration of 1,000 mg L^{-1} was recording the highest average height for seedlings, it reached 97.47 cm compared to the control treatment, which recorded the lowest seedling height of 82.67 cm.

As for spraying with Humic Acid, the same table results indicate a significant effect of spraying treatments on increasing seedlings height. The concentration of 2 g L^{-1} recorded the highest height of seedlings, which reached 95.33 cm compared to control treatment, which gave the lowest height, which reached 87.56 cm.

As for interaction factor between nano-NPK and humic acid, the same table results indicate a clear significant increase in orange seedlings height, the spraying treatment with nano-NPK at a concentration of 50 mg L^{-1} and humic acid with a concentration of 1 g L^{-1} recorded the highest seedling height of 95.00, which varies from spray treatment with nano-NPK at a concentration of 0 ml L^{-1} and humic acid at a concentration of 0 g L^{-1} recorded a seedling height of 78.00 cm.

Table (2) Effect of nano-NPK, humic acid, and their interaction on bitter orange seedlings height (cm)

Nano fertilizer concentrations NPK	Humic acid concentrations			Fertilization rate
	H0	H1	H2	
N0	78.00	81.67	88.33	82.67
N1	90.67	95.00	87.67	91.11
N2	94.00	88.33	110.00	97.47
Humic acid rate	87.56	88.33	95.33	
LSD 0.05				
N=0.962		H=0.962		N*H=1.666

2- Number of branches (seedling $branch^{-1}$):

The results in Table (3) indicate that spraying nano-NPK on bitter orange seedlings had a non-significant effect.

As for effect of spraying with humic acid, the same table results show significant effect of spraying treatments compared to control treatment, which gave the lowest average branches number, amounting to 8.22 seedling branches $^{-1}$, this increase was proportional to spray concentration increasing , as the treatment with a concentration of 2 g L^{-1} recorded the highest average branches number which reached 11.00 seedling branches $^{-1}$.

The results also show the effect of interaction treatments between nano-NPK and humic acid, as same table results indicate that there is no significant effect between the treatments.

Table (3) Effect of nano-NPK, humic acid, and the interaction between them on branches number of bitter orange seedlings (seedling $branch^{-1}$)

Nano fertilizer concentrations NPK	Humic acid concentrations			Fertilization rate
	H0	H1	H2	
N0	6.67	12.33	8.00	9.00
N1	8.33	10.67	13.00	10.67
N2	9.67	12.00	12.00	11.22

Humic acid rate	8.22	11.67	11.00	
LSD 0.05				
N=ns		H=1.857		N*H=ns

3- Total chlorophyll in leaves (mg $100g^{-1}$ fresh weight):

The results in Table (4) indicate a difference in bitter orange seedlings response when sprayed with different levels of nano-NPK in leaves chlorophyll content. The concentration exceeded 100 mg L^{-1}, giving it the highest chlorophyll content with a value 26.858 mg 100 g^{-1} fresh weight, while the lowest rate of leaves chlorophyll content when treated was 0 ml L^{-1} and amounted to 25.697 mg 100 g^{-1} fresh weight**.**

The results also showed that spraying bitter orange leaves with humic acid had a significant effect in increasing the chlorophyll content of the leaves, as the 2 g L^{-1} spraying treatment was characterized by giving it the highest chlorophyll content, which amounted to 26.696 mg 100 g^{-1} fresh weight, the treatment (without spraying) gave the lowest content of this trait and was 25.820 mg 100 g^{-1} fresh weight.

The interaction effect between the study factors varied in increasing the leaves chlorophyll content, as the treatment with 100 mg L^{-1} nano-NPK and 1 g L^{-1} humic acid giving the highest chlorophyll rate, which amounted to 27.133 mg 100 g^{-1} fresh weight compared to control treatment.

Table (4) Effect of nano-NPK and humic acid and their interaction on total chlorophyll in leaves of bitter orange seedlings (mg 100 g^{-1} fresh weight).

Nano fertilizer concentrations NPK	**Humic acid concentrations**			**Fertilization rate**
	H0	**H1**	**H2**	
N0	25.140	25.520	26.430	25.697
N1	25.767	26.000	26.760	26.179
N2	26.553	27.133	26.887	26.858
Humic acid rate	25.820	26.218	26.696	
LSD 0.05				
N=0.1118		H=0.1118		N*H=0.1937

4- Nitrogen in leaves (%):

It is clear from the results of Table (5) that spraying bitter orange seedlings with nano-NPK has a significant effect on nitrogen percentage of the leaves, the concentration 1 g L^{-1} NPK nanoparticle exceeds the rest treatments have the highest nitrogen percentage in the leaves, reaching 0.392%, while the nitrogen percentage in control treatment was 0.228%.

As for effect of spraying with humic acid on nitrogen content of leaves, table (5) results showed that there was a significant difference 2 g L^{-1} treatment was giving the highest percentage of nitrogen in leaves by 0.348%, while the lowest percentage was in control treatment it reached 0.245%.

The interaction between nano-NPK and humic acid has a significant effect on nitrogen percentage in leaves the results of table (5) showed that the treatment was superior to 100 mg L^{-1} nano-NPK and 1 g L^{-1} humic acid, and the highest percentage of nitrogen reached 0.440%, while it was the lowest the nitrogen percentage in the leaves during the control treatment reached 0.170%.

Table (5) Effect of nano-NPK, humic acid, and their interaction on nitrogen percentage in the leaves of bitter orange seedlings (%).

Nano fertilizer concentrations NPK	**Humic acid concentrations**			**Fertilization rate**
	H0	**H1**	**H2**	
N0	0.170	0.226	0.290	0.228
N1	0.233	0.280	0.353	0.288
N2	0.3330	0.440	0.403	0.392
Humic acid rate	0.245	0.315	0.348	
LSD 0.05				
N=0.01755		H=0.01755		N*H=0.03039

5-Potassium in leaves (%):

The results of Table (6) showed that there were significant differences in potassium percentage in bitter orange leaves when sprayed with nano-NPK and humic acid, the results showed the highest potassium percentage, reaching 1.691%, while the percentage was the lowest in orange seedlings leaves when treated without addition, it reached 1.378%.

The results in Table (6) indicated that there were significant differences between the treatments in potassium percentage in leaves when spraying with Humic acid, as 2 g L^{-1} treatment giving the highest percentage, reaching 1.621%, thus superior to control treatment, which gave 1.411. %.

As for interaction effect between the study factors, the results in the same table showed a significant increase in potassium percentage in leaves, spraying 50 mg L^{-1} nano NPK and 2 g L^{-1} humic acid resulted in the highest percentage of potassium, reaching 1.650%, while the lowest percentage was achieved when treated with 100 mg/L nano NPK and 1 g/L HUMC and reached 1.0786%.

Table (6) Effect of nano-NPK, humic acid, and their interaction on potassium percentage of bitter orange seedlings leaves (%)

Nano fertilizer concentrations NPK	Humic acid concentrations			Fertilization rate
	H0	H1	H2	
N0	1.280	1.360	1.496	1.378
N1	1.383	1.463	1.650	1.498
N2	1.570	1.0786	1.716	1.691
Humic acid rate	1.411	1.536	1.621	
LSD 0.05				
N=0.02796	H=0.02796	N*H=0.04843		

DISCUSSION:

Nutrition with Nano fertilizers plays an essential role in plant growth and development. In case of medicinal plants, increasing nutrients stimulates plant to increase growth and create essential oils and active substances.

It is noted from Tables (2-3-4) that the following studied vegetative characteristics (plant height, branches number, leaves number) outperformed with a direct increase with increasing nano fertilizer concentration, as a concentration of 100 mg L^{-1} gave the highest averages respectively (97.47 cm, 11.22 plant branches1). -26.858 mg 100 g $^{-1}$ fresh weight) compared to control treatment, which gave the lowest averages, this explains that nano fertilizer increases the plant's efficiency in absorbing water and nutrients, and that macronutrient nutrients have an effective role in various metabolic processes in the plant, thus increasing growth, which reflects positively on shoot activity, which leads to an increase in the characteristics of the shoot as a result of cell expansion due to growth hormones, this leads to an increase the branches number ,length, and leaves number, and this in turn is reflected in the remaining results, and these results are consistent with what was indicated by David (2020), this happened when fertilizing the pomegranate plant, as it was noted that nano fertilization led to an increase in the height and branches number compared to non-fertilized plants.

As for the significant increase that occurred in the percentage of both nitrogen and potassium in tables (5-6), which gave a concentration of 100 mg L^{-1} the highest average values (0.392%, 1.691%) compared to control treatment, the reason for mentioned characteristics increase is attributed to that nano fertilizers have a large surface area and their particles size is less than the pores found in plant leaves, which cause an increase in their penetration into plant tissues. Thus, the absorption of nutrients and elements is more efficient and smooth and contributed greatly to increasing leaves content, in addition to the fact that potassium element released by the leaves acts as a carrier for nitrate ions, as it transports them in KNO3 form, and this agrees with Shahian et al. (2019).

The increase in leaves number is also due to humic acid role in encouraging cell division and increasing their number, which is reflected in an increase in plant green growth and thus an increase in leaves number. Organic matter plays an important role in improving the chemical and physical properties of the soil and increasing the activity of microorganisms in it, which increases the availability of nutrients in it, which leads to increased plant growth (Gosh et al., 2004).

As a result of organic matter's content of essential nutrients such as nitrogen and phosphorus, it works to increase the plant's vegetative growth characteristics by increasing proteins formation, nucleic acids, and protoplasmic structure through the formation of RNA and DNA necessary for cell division, in addition to its role in carbon metabolism, respiration, and energy provision. ,conforms necessary for new cells formation, which increases plant growth (Zeiger and Taiz, 2006).

For the significant superiority in chlorophyll concentration, it is attributed to the fact that humic increases the availability of ready-made nutrients to the plant. It is compatible with plant growth, which increases chlorophyll formation and photosynthesis rate, and as a result, increases overall growth, the reason for increase elements in leaves due to adding humic acid to the soil increases the rates of Nitrogen released into the soil results in increased nitrogen accumulation in plants, as well as improving the physical and chemical properties of the soil and thus increasing leaves chlorophyll content (Letey Pang, 2000).

CONCLUSIONS:

1- Nano fertilizers use led to a significant increase in the shoots and their tolerance to different environmental conditions.

2- Nano fertilizers use has contributed to reducing the economic cost resulting from the use of regular fertilizer.
3- Adding different concentrations of humic acid led to an increase in the rate of vegetative and chemical growth in the plant.
4- The effect of interaction between the two factors of the study led to an increase in vegetative characteristics and chemicals.

RECOMMENDATIONS:

1- Paying attention to using nano-fertilizers compounded on other types of plants, at different concentrations and number of spraying times, and at different times during the growing season.
2- Pay attention to using foliar spraying of humic lion because it works to feed the leaves directly.
3- Introducing or experimenting with other varieties of crops and testing them in the conditions of the same region or other regions to determine which ones are best.

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